DOW CORNING

8EHQ-0702-15015 PIR 60 150

June 26, 2002

Document Control Office (7407)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Room G-099
Attn: TSCA Section 8(e) Coordinator
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Re: Supplemental Submission of Final Report to 8EHQ-01-15015
TSCA Section 8(e) Notification of Substantial Risk:
Skin Sensitization of Material 04005211 Using the Guinea Pig Maximization Test

Dear Sir:

In accordance with the provisions of Section 8(e) of the Toxic Substances Control Act (TSCA), as interpreted in the Statement of Interpretation and Enforcement Policy (40 FR 11110, 16 March 1978), Dow Corning is submitting the following recently issued final study report as a supplemental submission to our initial TSCA Section 8(e) notification of September 24, 2001 (8EHQ-01-15015).

Chemical Substance:

309263-22-7 Iodonium, (3-methylphenyl)phenyl-, ar'-C12-13-branched alkyl derivs., (OC-6-11)-hexafluoroantimonates(1-)

Manufacturer:

Dow Corning Corporation
2200 West Salzburg Road
Midland, Michigan 48686-0994

Contain NO CBI

8EHQ-01-15015

Final Study Report:

SKIN SENSITIZATION OF MATERIAL 04005211 USING THE GUINEA PIG MAXIMIZATION TEST

Dow Corning Corporation
2002-I0000-51342
March 25, 2002

8902000141

Dow Corning Corporation
Midland, Michigan 48686-0994
Phone: (989) 496-4000

Summary:

In a previous TSCA Section 8(e) submission, Dow Corning provided EPA with evidence of skin sensitization of material 04005211 upon completion of the in-life phase of an ongoing skin sensitization study using the Guinea Pig Maximization Test. Preliminary results indicated that the test article had the potential to cause strong skin sensitization. This preliminary result was based on all 20 test animals showing evidence of delayed contact hypersensitivity following exposure to the test article, as supplied, and at 50 percent (volume/ volume) in Alembicol D at the challenge application (study day 22). The final report confirms that material 04005211 produced evidence of skin sensitization in all 20 test animals and is considered to have the potential to cause skin sensitization.

Details:

Prior to the start of the study, a preliminary investigation was performed to identify (a) irritant test article concentrations suitable for the induction phase of the main study, (b) a maximum non-irritant concentration by the topical route of administration, and (c) a dilution of this, for the challenge phase. For the induction by intradermal injection (study day 1), the concentration of test article selected was 0.1% v/v in Alembicol D; and for induction by topical application (study day 8), the test article was administered as supplied.

Three pairs of intradermal injections (i.d.) were made at the clipped areas over the scapular region of male and female guinea pigs. The first pair of injections contained Freund's Complete Adjuvant (FCA) diluted with an equal volume of water for irrigation; the second pair of injections contained 0.1% v/v of the test article in Alembicol D; and the third pair of injections contained a 50:50 mixture of the 0.1% v/v concentration of the test article and FCA. Ten irritation control animals were also handled in a similar manner, but they were not exposed to the test article. The dermal reactions were observed and recorded 24 hours after induction of both the test and irritation control animals.

Six days following the intradermal induction, the same site was clipped and pretreated by gentle rubbing with 0.5 ml of 10% w/w sodium lauryl sulfate in petrolatum. Twenty-four hours later (study day 8), a 20 x 40 mm patch of Whatman No. 3 filter paper was saturated with approximately 0.4 ml of the test article, as supplied, then placed over the injection site. The filter paper was held in place with impermeable plastic adhesive tape, and further secured by elastic adhesive bandage wrapped around the torso of the animal. This dressing was left in place for 48 hours. The ten irritation control animals were treated in the same manner as the test animals, but they were not exposed to the test article. The

dermal reactions were observed and recorded 24 hours after the dressings were removed from both the test and irritation control animals.

On the day of challenge (study day 22), a 20 x 20 mm patch of Whatman No. 3 paper was saturated with either 0.2 ml of the test article, as supplied; 0.2 ml of a 50% v/v concentration of test article in Alembicol D; or 0.2 ml Alembicol D alone. All three patches were applied to the clipped left flank of the test and irritation control animals and held in place with impermeable plastic adhesive tape, and further secured by elastic adhesive bandage wrap around the torso of the animal. This dressing was left in place for 24 hours. The dermal reactions were observed and recorded 24, 48, and 72 hours after the dressings were removed from both the test and irritation control animals.

Dermal reaction (erythema/edema) in the test animals elicited by challenge application was compared with the findings obtained in the irritation control group. All 20 test animals showed evidence of delayed contact hypersensitivity following exposure to the test article, as supplied, and at 50% v/v in Alembicol D at the challenge application (study day 22). The test article was considered a strong sensitizer in guinea pigs.

Actions:

These results from the aforementioned study will be communicated to appropriate internal and external audiences.

If you have technical questions concerning this study, please contact Dr. Kathleen P. Plotzke, Director of Health and Environmental Sciences at 989-496-8046. If you require further general information regarding this submission, please contact Michael E. Thelen, Manager of U.S. EPA Regulatory Affairs, 989-496-4168 or at the address provided herein.

Sincerely,

Laura L. Perkins
Director of Environment, Health and Safety
(989) 496-8568

DOW CORNING CORPORATION
HEALTH & ENVIRONMENTAL SCIENCES
TECHNICAL REPORT

HUNTINGDON LIFE SCIENCES LTD
WOOLLEY ROAD
ALCONBURY
HUNTINGDON
CAMBRIDGESHIRE
PE28 4HS
ENGLAND

Report No.:	2002-I0000-51342
Title:	Skin Sensitisation of Material #04005211 using the Guinea Pig Maximization Test
DCC Study No.:	9568
External Testing Facility No.:	DCN 310/013925/SS
Test Substance:	Material #04005211
Study Director:	David G Coleman, B.Sc. (Hons).
Sponsor:	Dow Corning Corporation 2200 W. Salzburg Road Midland, MI 48686-0994 USA
Sponsor Representative:	Sharon L. Mudgett, B.S.
Testing Facility:	Huntingdon Life Sciences Ltd. Woolley Road Alconbury Huntingdon Cambridgeshire PE28 4HS ENGLAND
Study Completion Date:	March 25, 2002
Security Statement:	This report may be reproduced and shared with any Dow Corning employee. Distribution outside the corporation must be approved by the Director of H.E.S. When this INTERNAL report is no longer needed, it may be placed in office waste baskets for destruction.

TABLE OF CONTENTS

ABSTRACT

The study was designed to assess the skin sensitisation potential of material #04005211 using the guinea pig.

The procedure used is described in this report. The procedure complies with that described in the OECD Guideline for Testing of Chemicals No. 406 "Skin sensitisation" Adopted 17 July 1992.

The method used was the guinea pig maximization test described by MAGNUSSON, B. and KLIGMAN, A.M. (1970) *Allergic Contact Dermatitis in the Guinea pig: Identification of contact allergens*, Thomas, C.C., Springfield, Illinois, U.S.A.

Based on the results of a preliminary study and in compliance with the guideline, the following dose levels were selected:

Intradermal injection:	Material #04005211, 0.1% v/v in Alembicol D
Topical application:	Material #04005211, as supplied
Challenge application:	Material #04005211, as supplied and 50% v/v in Alembicol D

Twenty test and ten control guinea pigs were used in the main study and five control and five test animals were used in the positive control study.

In this study, material #04005211 produced evidence of skin sensitisation (delayed contact hypersensitivity) in all of the twenty test animals and is considered to have the potential to cause skin sensitisation.

Four of the five animals in the positive control group produced conclusive evidence of skin sensitisation following treatment with hexyl cinnamic aldehyde, thus demonstrating the validity of the method and sensitivity of the strain of animals used. The fifth animal gave an inconclusive response.

GLP COMPLIANCE STATEMENT

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and with the exceptions noted below I consider the data generated to be valid.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August, 1989.

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

The UK Good Laboratory Practice Regulations 1999, (Statutory Instrument No. 3106).

EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No L 77/8).

Samples of test substance and positive control mixtures were not taken for analysis. Determination of uniformity, concentration, physical and chemical stability of test substance and positive control mixtures in chosen vehicles was not determined at Huntingdon Life Sciences Ltd. Formulation concentrations can be verified by careful quantitative records made during preparation and administration.

The raw data has been reviewed by the Study Director, who certifies that the information contained in this report is consistent with and supported by the raw data.

D G Coleman

David G. Coleman, B.Sc. (Hons.),
Study Director,
Huntingdon Life Sciences Ltd.

25 March 2002

Date

Sharon L Mudgett

Sharon L. Mudgett, B.S.,
Sponsor Representative,
Dow Corning Corporation.

19-Mar-02

Date

QUALITY ASSURANCE STATEMENT

Study Title:	Skin Sensitisation Study of Material #04005211 using the Guinea Pig Maximization Test.
Huntingdon Life Sciences Study Number:	DCN/310
Study Director:	David G. Coleman, B.Sc.

This study has been audited by Huntingdon Life Sciences Ltd Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon.

Inspections were made by the Quality Assurance Department of various phases of the study conducted at Huntingdon and described in this report. The dates on which the inspections were made and the dates on which the findings were reported to the Study Director and to Management, Huntingdon Life Sciences Ltd are given below.

Study Phase	Date of Inspection	Findings reported to: Study Director & Management
Protocol audit	12 April 2001	12 April 2001
Experimental period: Topical Dose Administration	30 August 2001	30 August 2001
Report/data audit	16 November 2001	21 November 2001

Margaret Blows

Margaret Blows, MRQA,
Group Manager,
Department of Quality Assurance,
Huntingdon Life Sciences.

25 March 2002

Date

APPROVAL SIGNATURES

This report consists of Pages 1 through 32 including Tables 1-4 and Appendices 1 - 3.

Lynne A Waterson, BSc (Hons), I.D.T.,
Management,
Huntingdon Life Sciences Ltd.

25 March 2002
Date

David G. Coleman, B.Sc. (Hons.),
Study Director,
Short Term Studies Group,
Huntingdon Life Sciences Ltd.

25 March 2002
Date

Sharon L, Mudgett, B.S.
Sponsor Representative,
Dow Corning Corporation.

19-Mar-02
Date

STUDY INFORMATION

Study Initiation Date:	30 March 2001
Experimental Start Date:	14 August 2001
Experimental Termination Date:	18 September 2001
Study Completion Date:	25 March 2002
Study Director:	David G. Coleman, B.Sc.
Sponsor:	Dow Corning Corporation
Sponsor Representative:	Sharon L. Mudgett, B.S.
Senior Technician for the study:	Mark Egan
Animal facility manager – short term studies:	Dianne Cooper
Senior Toxicologist, Short Term Studies Group:	Lynne A. Waterson, B.Sc. (Hons.), I.D.T.
Director, Quality Assurance:	Roger W. Chapman, B.Sc.
Head, Department of Formulation Chemistry and Pharmacy:	Alan Anderson, B.Sc. (Hons.), C.Chem., FRSC
Head, Department of Microbiology:	John N. Carter, B.Sc. (Hons.)
Director, Laboratory Animal Sciences & Certificate holder:	David Whittaker, BVM&S, DLAS, MRCVS

I. INTRODUCTION

The study was designed to assess the skin sensitisation potential of the test substance using the guinea pig maximization test.

The procedure used is described in this report. The procedure complies with that described in the OECD Guideline for Testing of Chemicals No. 406 "Skin sensitisation" Adopted 17 July 1992.

The method used was the guinea pig maximization test described by MAGNUSSON, B. and KLIGMAN, A.M. (1970) *Allergic Contact Dermatitis in the Guinea pig: Identification of contact allergens*, Thomas, C.C., Springfield, Illinois, U.S.A.

The albino guinea pig (Dunkin /Hartley strain) was chosen as the test species as it had been shown to be a suitable model for this type of study and is the animal recommended in the test guideline.

The guinea pigs were dosed by intradermal injection and topical application as these are the routes of exposure required by the test guideline and method. Contact with the skin is an anticipated route of human exposure.

II. MATERIALS AND METHODS

A. Test Substance: Material #04005211, (supplied as Dow Corning® 2-7129 catalyst) batch reference number 16973-70, was received at Huntingdon Life Sciences on 25 July 2001, a reserve sample of material #04005211 was retained by the Sponsor. The test substance, a liquid, was stored at room temperature in the dark and was stable until 10 October 2002. The test substance, as received, is regarded as the "pure" material and representative of material #04005211. All the remaining test substance will be returned to the sponsor after the completion of all the relevant studies, with the exception of a 1ml sample which was retained by Huntingdon Life Sciences. The absorption of the test substance was not quantitated. Test substance characterisation has been carried out by the Sponsor (DCC Study number 9596).

Positive control material: The hexyl cinnamic aldehyde (HCA) used was a clear yellow liquid, batch number 01016AQ, expiry date 8 December 2001, purity 85%, stored at room temperature in the dark and supplied by Aldrich Chemical Company. A 1 ml archive sample was retained at Huntingdon Life Sciences.

B. Solubility trial: A solubility/miscibility trial with water was carried out, however as water was later found to be immiscible, a further vehicle trial using Alembicol D[1] was conducted. Material #04005211 was found to be miscible into Alembicol D forming a brown solution.

C. Animals: A stock supply of 45 male albino guinea pigs of the Dunkin/Hartley strain were purchased from David Hall, Darley Oaks Farm, Newchurch, Burton-on-Trent, Staffordshire, England. The guinea pigs for the main study were received from the supplier on 16 August 2001. On arrival, each animal was identified with a temporary number by ear tattoo. Following allocation to study, the animals were ear tagged with sequential permanent study numbers (control animals - 4101 to 4110, test animals - 4111 to 4130, control to positive control animals - 4131 to 4135 and positive control animals - 4136 to 4140). Throughout the pre-study period and during the study the guinea pigs were housed in cages of five in an isolated room. The guinea pigs were acclimatised for five days (see Deviations from protocol) during which time they were observed daily for signs of ill-health and the data reviewed by a veterinary officer before the animals were selected for study. In addition, during the study, each cage was identified by a coloured label displaying but not limited to the study number, animal numbers and initials of the Study Director and Home Office licensee. An additional stock of ten male albino guinea pigs of the same strain from the same supplier were used for preliminary investigations.

D. Food and Water: The guinea pigs were provided, with a vitamin C enriched guinea pig diet, (9600 FD2 SQC (supplier: Harlan Teklad, Shaw's Farm, Blackthorn, Bicester, Oxon, England)) and drinking water (supplier: Anglian Water Services Ltd). Both diet and drinking water were provided *ad libitum* using diet hoppers and water bowls in each cage. No contaminants capable of adversely affecting the integrity or interpretation of the results from this study were known to be present in the basal diet or the drinking water during the conduct of this study. Certificates of analysis pertaining to the diet and water used on this study were reviewed by the Study Director. Autoclaved hay was given to the guinea pigs thrice weekly (supplier: R S Biotech, Tower Works, Well St, Finedon, Northants). Provision of hay is standard practice at this laboratory and is not considered to have any influence on test results interpretation.

E. Housing and Environment: The guinea pigs were housed in groups of five in suspended plastic cages with solid floors and sawdust bedding. The internal cage dimensions are 75 cm wide, 55 cm deep, 25 cm high (floor area 4125 cm^2). The cage size is in compliance with UK Animal Welfare Guidelines. Thermostatic controls were set to maintain a temperature of approximately 21 °C (target range for study 21°C ± 2). Relative humidity was not fully controlled but was expected to be in the range 30 - 70%. Temperature and humidity were recorded continuously using a seven day circular chart recorder. Permanent daily recordings of these parameters were made and these are archived with other Departmental raw data. Air exchange was maintained at approximately 15 air changes per hour and lighting was controlled by means of a time switch to

[1] Alembicol D, batch – ALD1036, expiry – 19.01.02, appearance - clear liquid, storage conditions - room temperature, supplied by Alembic Products Ltd, Chester, CH4 8RQ

provide 12 hours of artificial light (0600 - 1800 hours GMT) in each 24-hour period. For environmental enrichment, a plastic tubular pipe was included in the cage – this is standard practice at this laboratory and is not considered to have any influence on the test system.

F. Methods:

1. Animals: The stock supply of forty five healthy male albino guinea pigs of the Dunkin/Hartley strain were randomly allocated to study groups by bodyweight so that the group mean bodyweights were approximately equalized (using a computer program). Animals were approximately 5 to 8 weeks of age and in the bodyweight range 351g to 447g prior to dosing on Day 1 of the study.

2. Animal welfare: This study complied with all applicable sections of the Animals (Scientific Procedures) Act 1986 of the United Kingdom and the associated Code of Practice for the Housing and Care of Animals used in Scientific Procedures issued under Section 21 of the Act. As required by condition 6 of the Project Licence issued under the Act, the procedures used in this study were designed to avoid or minimise discomfort, distress and pain to animals.

3. Test substance preparation: When not administered as supplied, the test substance mixtures were prepared on the day of dosing in Alembicol D. When formulated, the doses were administered within two hours of preparation. The absorption of the test substance was not quantitated. The positive control material was either prepared on the day of dosing in Alembicol D or administered as supplied (neat).

4. Preliminary investigation:

   The intradermal and topical irritancy of a range of dilutions of the test substance was investigated to identify where possible (a) irritant test substance concentrations suitable for the induction phase of the main study and (b) a maximum non-irritant concentration by the topical route of administration and a dilution of this for the challenge phase.

   Animals for the topical irritancy investigations were pre-treated with an intradermal injection of Freund's Complete Adjuvant (FCA), 50 : 50 with water for irrigation (Ph.Eur.), seven days prior to the start of the preliminary investigations.

   The numerical values given to the dermal reactions observed in the preliminary tests are shown in Appendix 2.

The animals for the preliminary investigations were clipped and shaved approximately 24 hours prior to dosing and for the irritancy investigations, the animals were reshaved prior to dosing on the day of dosing. The procedure employed for these investigations was as follows:

Intradermal injections - A range of concentrations of the test substance (0.05 to 50% v/v) in a Alembicol D were injected intradermally (0.1 ml/site) into the clipped scapular region of four guinea pigs (two per concentration) using a 1 ml syringe and 26 G needle. The resulting dermal responses were assessed approximately 24, 48 and 72 hours later.

Topical application - Patches of Whatman No. 3 paper (20 mm x 20 mm) were saturated (volume approximately 0.2 ml) with a range of concentrations of the test substance (25% v/v to as supplied) in Alembicol D and applied to the clipped and shaved flanks of each of four guinea pigs. The patches were covered by a strip of "Blenderm" and firmly secured by "Elastoplast" wound round the trunk and fixed an impervious plastic adhesive tape. The dressings were removed after an exposure period of approximately 24 hours and the reaction sites were assessed for erythema and oedema (reported as 0 hours in Appendix 2). Further examination of the sites was carried out approximately 24 and 48 hours after removal of the dressings.

No irritation was observed following the first preliminary topical application, however, severe reactions were observed following the induction topical application on the main study. Therefore to ensure the test material had not changed in irritancy since the initial preliminary investigations, an additional investigation was conducted. Two guinea-pigs were treated with FCA 3 days prior to dosing and these animals were treated in a similar manner using the same levels as the initial preliminary investigations.

Selection of concentrations of test substance for the main study

Based on the results of the preliminary investigations, the following concentrations of material #04005211 were selected:

Induction intradermal injection – 0.1% v/v in Alembicol D. This was the highest concentration that caused irritation but did not adversely affect the animals.

Induction topical application - As supplied.

Topical challenge - As supplied and 50% v/v in Alembicol D. For preliminary investigations the test material applied topically as supplied by the Sponsor did not give rise to irritating effects.

Based on historical data the concentrations of hexyl cinnamic aldehyde (HCA) used in the positive control study are as follows:

Induction intradermal injection:	HCA, 10% v/v in Alembicol D
Induction topical application:	HCA, as supplied
Topical challenge:	HCA, as supplied and 50% v/v in Alembicol D

5. Main study:

The procedure may be considered in two parts, Induction and Challenge.

Induction intradermal injections - test animals

An approximately 40 × 60 mm area of dorsal skin on the scapular region of the guinea pig was clipped free of hair with electric clippers on the day prior to the start of the study. On Day 1 of the study three pairs of intradermal injections (0.1 ml/site) were made into a 20 × 40 mm area within the clipped area using a 1 ml syringe and a 26G needle.

Injectables for the test animals were prepared as follows:

1. Freund's Complete Adjuvant[2] was diluted with an equal volume of water for irrigation[3] (Ph.Eur.).
2. Material #04005211, 0.1% v/v in Alembicol D.
3. Material #04005211, 0.1% v/v in a 50 : 50 mixture of Freund's Complete Adjuvant and Alembicol D.

Injectables for the control animals were prepared as follows:

1. Freund's Complete Adjuvant was diluted with an equal volume of water for irrigation (Ph.Eur.).
2. Alembicol D.
3. 50 : 50 mixture of Freund's Complete Adjuvant and Alembicol D.

Injectables for the positive control animals were prepared as follows

1. Freund's Complete Adjuvant was diluted with an equal volume of water for irrigation (Ph.Eur.).
2. HCA, 10% v/v in Alembicol D.
3. HCA, 10% v/v in A 50:50 mixture of FCA and Alembicol D.

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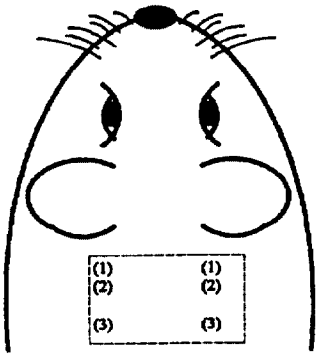
[2] FCA, batch 20K8933, expiry 18.07.2006, supplier: Sigma, UK.

[3] Water for irrigation, batch 01D11B25, expiry March 2004, supplier: Baxter Healthcare, Thetford, Norfolk, UK.

Position of intradermal injections and induction topical application



Induction topical application - test animals

The preliminary investigations indicated that the neat test substance caused no irritation when applied topically. Therefore, five days after the injections (Day 6), the same 40 × 60 mm interscapular area was clipped and shaved free of hair and on Day 7, the site was pre-treated by gentle rubbing with 0.5 ml per site of 10% w/w sodium lauryl sulphate[4] in petrolatum[5]. The next day (Day 8, seven days after intradermal injections) a 20 × 40 mm patch of Whatman No. 3 paper was saturated with approximately 0.4 ml of material #04005211, as supplied. The patch was placed over the injection sites on the skin of the interscapular region of the test animals and covered by a length of impermeable plastic adhesive tape (50 mm width "Blenderm"). This in turn was firmly secured by elastic adhesive bandage (50 mm width "Elastoplast") wound round the torso of the animal and fixed with an impervious plastic adhesive tape. The dressing was left in place for 48 hours.

Induction - control animals

During the induction phase, the control animals were treated similarly to the test animals with the exception that the test substance was omitted. As the test material was applied topically to the test group as supplied, the control animals received a dry patch of Whatman paper.

The dermal reactions observed after each induction phase in both control and test animals were recorded by group and are given in Table 1. This consisted of a descriptive assessment of the intensity of the response. These observations were made approximately 24 hours following the intradermal induction and following removal of the dressings for the topical induction.

[4] Sodium lauryl sulphate (SLS), batch 0090035460, expiry 26/03/2006, supplier: Fisher Chemicals, England

[5] Petrolatum, batch BN043T, expiry 06/08/2003, supplier: Chesebrough Ponds, UK.

Induction – positive control group

During the induction phase, the positive control animals were treated similarly to the test animals with the exception that the test substance was replaced with 10% v/v HCA in Alembicol D for the intradermal injections and as supplied for the topical application.

The dermal reactions observed after each induction phase in the positive control group were recorded and are given in Table 3.

6. Challenge - control and test animals

The control and test animals were challenged topically two weeks after the topical induction application using material #04005211, as supplied and 50% v/v in Alembicol D.

Hair was removed by clipping and then shaving from an area on the left flank of each guinea pig the day prior to the challenge application and reshaved just prior to dosing. A 20 × 20 mm patch of Whatman No. 3 paper was saturated with approximately 0.2 ml of material #04005211, as supplied and applied to an anterior site on the flank. Material #04005211, 50% v/v in Alembicol D was applied in a similar manner to a middle site and the vehicle alone (Alembicol D) was applied to a posterior site. The patches were sealed to the left flank for 24 hours under strips of "Blenderm" covered by "Elastoplast" wound round the trunk and secured with an impervious plastic adhesive tape.

The dermal reactions observed after the challenge phase in the control and test animals were recorded and are given in Table 2.

Challenge - Positive control group

During the challenge application, the positive control animals were treated similarly to the test animals with the exception that the test substance was replaced with HCA, as supplied for the anterior site and 50% v/v HCA in Alembicol D for the middle site. The posterior site received the vehicle (Alembicol D) alone.

The dermal reactions observed after the challenge phase in the positive control group were recorded and are given in Table 4.

7. Clinical signs: All animals were observed daily for signs of ill health or toxicity. For the main study, a positive notation was made for each animal on each dosing occasion (Days 1, 8 and 22).

8. Dermal responses: The dermal reactions resulting from intradermal injection and topical application on the preliminary study, and topical application at the challenge were assessed using the following arbitrary numerical system (based on Draize JH, Appraisal of the Safety of Chemicals in Foods, Drugs & Cosmetics, Assoc. Food & Drug Officials of the US, Austin, TX; 1959):

Erythema and eschar formation:

No erythema	0
Slight erythema	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

Oedema formation:

No oedema	0
Slight oedema	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

Other lesions:

N	Necrosis
#	Blanching of dose site
NE	Necrotic edge
NP	Necrotic patch

The challenge sites were evaluated 'blind' (*ie* the scorer not knowing the identity of the animal) 24, 48 and 72 hours after removal of the patches.

9. Bodyweight: The bodyweight of each guinea pig on the main study was recorded on Day 1 (day of intradermal injections) and the day after the last observation was made to the challenge application (see Deviations from protocol).

10. Termination: Following the last day of dermal observations, all animals were humanely sacrificed *via* an intraperitoneal injection of sodium pentobarbitone 'Euthatal'.

11. Interpretation of the results: Dermal reactions in the test animals elicited by the challenge application were compared with the findings simultaneously obtained in the control animals.

    A test animal was considered to show positive evidence of delayed contact hypersensitivity if the observed dermal reaction at challenge was definitely more marked and/or persistent than the maximum reaction seen in animals of the control group.

    If the dermal reaction seen in a test animal at challenge was slightly more marked and/or persistent than (but not clearly distinguishable from) the maximum reaction seen in control animals, the result for that test animal was classified as inconclusive.

A test animal was considered to show no evidence of delayed contact hypersensitivity if the dermal reaction resulting from the challenge application was the same as, or less marked and/or persistent than the maximum reaction seen in animals of the control group.

As a sensitisation reaction was not induced classification using the scoring rating of Kligman (Kligman A.M., *J. Invest. Dermatology* 1966), was not required.

G. Location of Study records: The protocol and all the amendments as well as all raw data, a sample of the test substance and study related documents generated during the course of the study at Huntingdon Life Sciences Ltd., together with a copy of the original final report are lodged in the Huntingdon Life Sciences Ltd., Archive, Huntingdon, England. Such records will be retained for a minimum period of ten years from the date of issue of the final report. At the end of the ten year retention period the client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the client's prior approval.

H. Statistical analysis: None

I. References:

EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No L 77/8).

EPA Health Effects Testing Guidelines, OPPTS 870.260 Skin sensitisation EPA 712-C-98-197, August 1998.

MAGNUSSON, B. and KLIGMAN, A.M. (1970) *Allergic Contact Dermatitis in the Guinea pig: Identification of contact allergens*, Thomas, C.C., Springfield, Illinois, U.S.A.

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

The UK Good Laboratory Practice Regulations 1999, (Statutory Instrument No. 3106).

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August, 1989.

III. RESULTS

A. Mortality and Clinical Signs: There were no unscheduled deaths and no signs of ill health or toxicity were recorded.

B. Body Weights: Bodyweight increases were recorded for all guinea pigs over the period of the study. Individual bodyweights are shown in Appendix 1.

C. Induction: Dermal reactions seen following the induction applications are summarised in Table 1 (test and control animals) and Table 3 (positive control animals).

a) Intradermal injections: Necrosis was recorded at sites receiving Freund's Complete Adjuvant in test and control animals. Slight irritation was seen in 11 of the twenty test animals at sites receiving material #04005211, 0.1% v/v in Alembicol D and slight irritation was observed in 7 of the ten control animals receiving Alembicol D.

Slight irritation was seen in four of the five positive control animals receiving HCA, 10% v/v in Alembicol D and slight irritation was observed in all control animals receiving Alembicol D.

b) Topical application: Severe erythema and blanching was observed in all of the test animals following topical application with material #04005211 as supplied and no erythema was seen in any of the control guinea pigs.

Well-defined erythema was observed in all positive control animals following topical application with HCA as supplied, with no erythema observed in any of the control animals.

D. Challenge: The numerical values given to the dermal reactions elicited by the challenge applications with material #04005211 are shown in Table 2 (test and controls) and Table 4 (positive control). The dermal reactions observed in all of the twenty test animals were more marked and persistent than those seen in the control animals, therefore all twenty test animals gave positive responses.

The dermal reactions noted for four of the five positive control animals were generally more marked and persistent than those seen for controls. Therefore four of the five positive control animals produced evidence of skin sensitisation. The dermal reactions observed for the fifth animal were similar to those observed in the controls, therefore this animal gave an inconclusive response. The ability of the positive control substance to provoke a response demonstrates the sensitivity and reliability of the experimental technique as well as the stability of the positive control substance and/or its formulation in the vehicle.

E. Deviations from protocol: The induction dose site was re-clipped and re-shaved five days after the intradermal injections (Day 6) rather than on Day 7 as protocol. The animals were weighed 1 day after the last day observations to the challenge application and not on the last day of observations as stated in the protocol. A 1 ml (rather than 1 g) sample of test material was taken and retained by Huntingdon Life Sciences. The main study guinea-pigs were acclimatized for five days prior to allocation to the study (rather than seven days as protocol). To comply with US EPA Good laboratory Practice guidelines, the raw data will be archived for a minimum period of 10 years (not 5 years as protocol). There were no other deviations from the protocol.

IV. CONCLUSION

Under the conditions of this study, material #04005211 produced evidence of skin sensitisation (delayed contact hypersensitivity) in all of the test animals.

V. AMENDMENTS TO PROTOCOL

Following finalisation of the protocol, protocol amendments were required for the following reasons:

Protocol amendment No. 1:

To reschedule the study following the arrival of a new sample of material at Huntingdon Life Sciences, as the study was postponed at the request of the Sponsor due to the original sample of test material having a low pH.
To insert the details of the new sample of test material and the details of the hexyl cinnamic aldehyde.
To correct a protocol inconsistency in the Day numbers of the induction application and the challenge application.
To correct the SOP reference for the cleaning of cages.
To update the date of the COSHH regulations.
To remove the requirement for six monthly microbial analysis of the drinking water at source.

Protocol amendment No. 2:

To state the concentrations and vehicle used on the main study, based on information provided in the preliminary investigations.

Protocol amendment No. 3:

To conduct an additional topical irritancy preliminary investigation to ensure that the test material has not changed in irritancy since the initial preliminary investigations, as the test material administered as supplied did not elicit any dermal irritation during the initial preliminary topical irritancy investigations. However, when the material was administered as supplied for the induction topical application, severe erythema, with blanching was observed. Furthermore well-defined to moderate dermal reactions were observed in the rabbit skin irritation study with the same material (HLS report number DCN 313/013971/SE).

VI. TABLE 1

Dermal reactions observed after each induction

Group	Animal number	Intradermal injections Site number			Topical application
		1	2	3	
Control	4101	N	1	N	0
	4102	N	0	N	0
	4103	N	1	N	0
	4104	N	1	N	0
	4105	N	1	N	0
	4106	N	1	N	0
	4107	N	1	N	0
	4108	N	0	N	0
	4109	N	0	N	0
	4110	N	1	N	0
Test	4111	N	1	N	4 #
	4112	N	1	N	4 #
	4113	N	1	N	4 #
	4114	N	0	N	4 #
	4115	N	0	N	4 #
	4116	N	1	N	4 #
	4117	N	0	N	4 #
	4118	N	1	N	4 #
	4119	N	1	N	4 #
	412.0	N	1	N	4 #
	4121	N	0	N	4 #
	4122	N	0	N	4 #
	4123	N	0	N	4 #
	4124	N	0	N	4 #
	4125	N	1	N	4 #
	4126	N	1	N	4 #
	4127	N	1	N	4 #
	4128	N	0	N	4 #
	4129	N	0	N	4 #
	4130	N	1	N	4 #

Intradermal injections
Control animals: See P 12
Test animals: See P12

N Necrosis
0 No irritation
1 Slight irritation
2 Well-defined irritation
3 Moderate irritation
4 Severe irritation

Topical application
Control animals: Dry patch
Test animals: Material #04005211, as supplied

0 No erythema
1 Slight erythema
2 Well-defined erythema
3 Moderate erythema
4 Severe erythema
Blanching of the dose site

VI. TABLE 2

Dermal reactions observed after the challenge application
Test Substance

Freund's treated controls

Guinea pig number	E = Erythema O = Oedema	Score								
		24 Hours			48 Hours			72 Hours		
		A	M	P	A	M	P	A	M	P
4101	E	1	1	0	2	1	0	2	2	0
	O	1	1	0	1	1	0	2	2	0
4102	E	2	1	0	2	1	0	2	2	0
	O	1	1	0	2	1	0	2	2	0
4103	E	2	2	0	2 NE	1	0	3 NE	3	0
	O	1	1	0	2	1	0	3	2	0
4104	E	2	1	0	2	2	0	3 NP	3 NP	0
	O	1	1	0	2	2	0	3	2	0
4105	E	0	0	0	2	1	0	2	2	0
	O	0	0	0	2	1	0	2	2	0
4106	E	2	2	0	2	1	0	3 NP	2	0
	O	2	2	0	2	1	0	3	1	0
4107	E	2	0	0	2	2	0	3 NP	3 NP	0
	O	1	1	0	2	2	0	3	3	0
4108	E	1	1	0	2	2	0	2	2	0
	O	1	0	0	1	1	0	2	2	0
4109	E	2	2	0	2	2	0	3	2	0
	O	2	2	0	2	2	0	2	2	0
4110	E	2	1	0	1	1	0	2 *	2	0
	O	2	1	0	1	1	0	2	2	0

A Anterior site, exposed to material #04005211, as supplied
M Middle site, exposed to material #04005211, 50% v/v in Alembicol D
P Posterior site, exposed to vehicle (Alembicol D)
NP Necrotic patch
NE Necrotic edge
* Dryness and sloughing of the epidermis

VI. TABLE 2 (continued)

Dermal reactions observed after the challenge application
Test Substance

Test animals

Guinea pig number	E = Erythema O = Oedema	Score									Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours			48 Hours			72 Hours			
		A	M	P	A	M	P	A	M	P	
4111	E	2	2	0	2 NP	2 NP	0	4 NP	N	0	+
	O	2	2	0	3	2	0	3	4	0	
4112	E	3	2 NP	0	2	2 NP	0	N	N	0	+
	O	2	2	0	2	2	0	3	4	0	
4113	E	2	2	0	2	2 NP	0	N	N	0	+
	O	2	2	0	3	3	0	4	4	0	
4114	E	3 NP	3 NP	0	3 NP	2 NP	0	N	N	0	+
	O	3	3	0	3	3	0	4	4	0	
4115	E	2	2	0	2	2	0	N	N	0	+
	O	3	3	0	2	2	0	3	3	0	
4116	E	2 NP	2	0	2	2 NP	0	N	N	0	+
	O	2	3	0	3	3	0	4	4	0	
4117	E	2	2	0	2	2	0	N	N	0	+
	O	2	2	0	2	2	0	4	4	0	
4118	E	3	3	0	2	2 NP	0	N	N	0	+
	O	2	2	0	2	3	0	4	4	0	
4119	E	2	2	0	1	2	0	N	N	0	+
	O	2	2	0	1	2	0	4	4	0	
4120	E	2	2	0	2	1	0	N	N	0	+
	O	2	1	0	2	1	0	4	4	0	

A Anterior site, exposed to material #04005211, as supplied
M Middle site, exposed to material #04005211, 50% v/v in Alembicol D
P Posterior site, exposed to vehicle (Alembicol D)
NP Necrotic patch
N Necrosis

VI. TABLE 2 (continued)

Dermal reactions observed after the challenge application
Test Substance

Test animals

Guinea pig number	E = Erythema O = Oedema	Score									Results Positive (+) Negative (-) Inconclusive (±)
		24 Hours			48 Hours			72 Hours			
		A	M	P	A	M	P	A	M	P	
4121	E	2	2	0	2	2	0	N	N	0	+
	O	2	2	0	3	3	0	4	4	0	
4122	E	2	2	0	2	2	0	N	N	0	+
	O	2	2	0	2	2	0	4	4	0	
4123	E	2	2	0	2 NE	2 NE	0	N	N	0	+
	O	3	2	0	2	2	0	4	4	0	
4124	E	2 NP	2 NP	0	2 NP	2 NP	0	N	N	0	+
	O	3	2	0	3	2	0	4	4	0	
4125	E	2 NP	2 NP	0	2 NP	2 NP	0	N	N	0	+
	O	3	2	0	2	2	0	4	4	0	
4126	E	2	1	0	2	1	0	N	N	0	+
	O	2	0	0	2	2	0	4	4	0	
4127	E	2 NP	2 NP	0	2 NP	2 NP	0	N	N	0	+
	O	2	3	0	3	3	0	4	4	0	
4128	E	2 NP	2 NP	0	2 NP	2 NP	0	N	N	0	+
	O	2	2	0	2	2	0	4	4	0	
4129	E	2	2	0	2	2	0	N	N	0	+
	O	2	2	0	2	2	0	4	4	0	
4130	E	2 NP	2 NP	0	2 NP	2 NP	0	N	N	0	+
	O	3	3	0	3	3	0	4	4	0	

A Anterior site, exposed to material #04005211, as supplied
M Middle site, exposed to material #04005211, 50% v/v in Alembicol D
P Posterior site, exposed to vehicle (Alembicol D)
NP Necrotic patch
NE Necrotic edge
N Necrosis

VI. TABLE 3

Dermal reactions observed after each induction (positive control)

Group	Animal number	Intradermal injections Site number			Topical application
		1	2	3	
Control	4131	N	1	N	0
	4132	N	1	N	0
	4133	N	1	N	0
	4134	N	1	N	0
	4135	N	1	N	0
Test	4136	N	1	N	2
	4137	N	1	N	2
	4138	N	0	N	2
	4139	N	1	N	2
	4140	N	1	N	2

Intradermal injections

Control animals: See P 12
Test animals: See P 12

N Necrosis
0 No irritation
1 Slight irritation
2 Well-defined irritation
3 Moderate irritation
4 Severe irritation

Topical application

Control animals: Dry patch
Test animals: HCA, as supplied

0 No erythema
1 Slight erythema
2 Well-defined erythema
3 Moderate erythema
4 Severe erythema

VI. TABLE 4

Dermal reactions observed after the challenge application
Positive Control

Freund's treated controls

Guinea pig number	E = Erythema O = Oedema	Score								
		24 hours			48 hours			72 hours		
		A	M	P	A	M	P	A	M	P
4131	E	0	0	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0	0	0
4132	E	0	0	0	1	0	0	0	0	0
	O	0	0	0	0	0	0	0	0	0
4133	E	1	0	0	1	0	0	0	0	0
	O	1	0	0	1	0	0	0	0	0
4134	E	0	0	0	0	0	0	0	0	0
	O	0	0	0	0	0	0	0	0	0
4135	E	1	0	0	0	0	0	0	0	0
	O	1	0	0	0	0	0	0	0	0

A Anterior site, exposed to hexyl cinnamic aldehyde, as supplied
M Middle site, exposed to hexyl cinnamic aldehyde, 50% v/v in Alembicol D
P Posterior site, exposed to vehicle (Alembicol D)

VI. TABLE 4 (continued)

Dermal reactions observed after the challenge application
Positive Control

Test animals

Guinea pig number	E = Erythema O = Oedema	Score									Results Positive (+) Negative (-) Inconclusive (±)
		24 hours			48 hours			72 hours			
		A	M	P	A	M	P	A	M	P	
4136	E	1	1	0	1	0	0	1	0	0	+
	O	0	0	0	1	0	0	0	0	0	
4137	E	1	1	0	1	1	0	1	1	0	+
	O	0	2	0	1	1	0	1	0	0	
4138	E	1	0	0	1	1	0	1	0	0	+
	O	0	0	0	1	0	0	1	0	0	
4139	E	1	1	0	0	0	0	1	0	0	±
	O	1	1	0	0	0	0	0	0	0	
4140	E	2	1	0	2	1	0	1	0	0	+
	O	2	1	0	1	1	0	1	0	0	

A Anterior site, exposed to hexyl cinnamic aldehyde, as supplied
M Middle site, exposed to hexyl cinnamic aldehyde, 50% v/v in Alembicol D
P Posterior site, exposed to vehicle (Alembicol D)

VII. APPENDIX 1

Individual bodyweights (g)

Main study

Group	Guinea pig number	Day 1 23 August 2001	Pre-terminal 18 September 2001
Control	4101	403	670
	4102	437	688
	4103	398	643
	4104	384	551
	4105	393	564
	4106	420	568
	4107	414	604
	4108	423	685
	4109	395	661
	4110	407	601
Test	4111	433	613
	4112	402	545
	4113	396	606
	4114	423	508
	4115	429	612
	4116	351	540
	4117	380	506
	4118	402	598
	4119	411	597
	4120	431	670
	4121	433	533
	4122	429	605
	4123	415	568
	4124	400	562
	4125	390	570
	4126	373	625
	4127	379	555
	4128	420	599
	4129	407	648
	4130	441	664

VII. APPENDIX 1 (continued)

Individual bodyweights (g)

Positive control group

Group	Guinea pig number	Day 1 23 August 2001	Pre-terminal 18 September 2001
Control	4131	447	693
	4132	403	663
	4133	400	670
	4134	406	686
	4135	386	587
Positive control	4136	412	682
	4137	425	654
	4138	390	613
	4139	428	668
	4140	379	633

VII. APPENDIX 2

Results of preliminary investigations with material #04005211

Intradermal injections

Vehicle: Alembicol D

Guinea pig number	Concentration % v/v	Hours	Score 24	48	72
700	50	D	9	10	10
		E	N	N	N
		O	2	2	2
	25	D	11	11	11
		E	N	N	N
		O	2	2	2
	10	D	10	10	10
		E	N	N	N
		O	2	2	2
	5	D	11	10	10
		E	N	N	N
		O	2	2	2
	Vehicle control	D	9	9	9
		E	2	2	2
		O	2	2	1

Guinea pig number	Concentration % v/v	Hours	Score 24	48	72
701	50	D	10	10	10
		E	N	N	N
		O	2	2	2
	25	D	10	10	10
		E	N	N	N
		O	2	2	2
	10	D	10	10	10
		E	N	N	N
		O	2	2	2
	5	D	10	10	10
		E	N	N	N
		O	2	2	2
	Vehicle control	D	8	8	8
		E	2	2	2
		O	2	2	1

Key:

D Diameter (mm)
E Erythema (0 - 4 numerical scores)
O Oedema (0 - 4 numerical scores)
N Necrosis

The approximate diameter (mm) of the dermal response at the intradermal injection sites was recorded in the preliminary study only to assist in the choice of concentrations for the main study.

VII. APPENDIX 2 (continued)

Results of preliminary investigations with material #04005211

Intradermal injections

Vehicle: Alembicol D

Guinea pig number	Concentration % v/v	Hours	Score 24	48	72
702	1	D	10	10	10
		E	N	N	N
		O	2	2	2
	0.5	D	10	10	10
		E	N	N	N
		O	2	2	2
	0.1	D	8	8	8
		E	2	2	2
		O	2	2	2
	0.05	D	8	8	7
		E	2	2	2
		O	2	2	2
	Vehicle control	D	8	8	7
		E	2	2	2
		O	2	2	2

Guinea pig number	Concentration % v/v	Hours	Score 24	48	72
703	1	D	10	10	10
		E	N	N	N
		O	2	2	2
	0.5	D	10	10	10
		E	N	N	N
		O	2	2	2
	0.1	D	9	8	8
		E	2	2	2
		O	2	2	2
	0.05	D	9	8	8
		E	2	2	2
		O	1	1	1
	Vehicle control	D	8	8	7
		E	2	2	2
		O	1	1	1

Key:

D Diameter (mm)
E Erythema (0 - 4 numerical scores)
O Oedema (0 - 4 numerical scores)
N Necrosis

The approximate diameter (mm) of the dermal response at the intradermal injection sites was recorded in the preliminary study only to assist in the choice of concentrations for the main study.

VII. APPENDIX 2 (continued)

Results of preliminary investigations with material #04005211

Topical application

Vehicle: Alembicol D

Guinea pig number	Location of site		Concentration % v/v	Score					
				0 Hours		24 Hours		48 Hours	
				E	O	E	O	E	O
704	LF	TS	As supplied	0	0	0	0	0	0
		BS	75	0	0	0	0	0	0
	RF	TS	50	0	0	0	0	0	0
		BS	25	0	0	0	0	0	0
705	LF	TS	As supplied	0	0	0	0	0	0
		BS	75	0	0	0	0	0	0
	RF	TS	50	0	0	0	0	0	0
		BS	25	0	0	0	0	0	0
710	LF	TS	As supplied	0	0	0	0	0	0
		BS	75	0	0	0	0	0	0
	RF	TS	50	0	0	0	0	0	0
		BS	25	0	0	0	0	0	0
711	LF	TS	As supplied	0	0	0	0	0	0
		BS	75	0	0	0	0	0	0
	RF	TS	50	0	0	0	0	0	0
		BS	25	0	0	0	0	0	0
90†	LF	TS	As supplied	0	0	0	0	0	0
		BS	75	0	0	0	0	0	0
	RF	TS	50	0	0	0	0	0	0
		BS	25	0	0	0	0	0	0
91†	LF	TS	As supplied	0	0	0	0	0	0
		BS	75	0	0	0	0	0	0
	RF	TS	50	0	0	0	0	0	0
		BS	25	0	0	0	0	0	0

E Erythema (0 - 4 numerical scores)
O Oedema (0 - 4 numerical scores)

LF Left flank
RF Right flank
TS Top site
BS Bottom site
† Results of additional investigations conducted to check the irritancy of material #04005211 (Protocol amendment No. 3)

VII. APPENDIX 3

Huntingdon Research Centre GLP Compliance Statement 2001

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT
OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY	TEST TYPE
Huntingdon Life Sciences Huntingdon Research Centre Wooley Road Alconbury Huntingdon Cambs. PE28 4HS	Analytical Chemistry Clinical Chemistry Ecosystems Environmental Fate Environmental Toxicity Phys/Chem Testing Toxicology

DATE OF INSPECTION
15th January 2001

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

Roger G. Alexander
3/4/01

Dr. Roger G. Alexander
Head, UK GLP Monitoring Authority